Atmospheric NH3 deposition, S and N metabolism in curly kale

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Chapter 5.

Impact of atmospheric NH_3 deposition on plant growth and functioning – a case study with *Brassica oleracea* L.

Abstract

Brassica oleracea L. (curly kale) was exposed to 0, 2, 4, 6 and 8 µl l^{-1} NH_3 during one week and the impact on growth and N compounds was determined. Exposure to NH_3 increased shoot biomass production at 2 and 4 µl l^{-1}, but resulted in an inhibition of shoot and root growth at 6 and 8 µl l^{-1}. Shoot to root ratio was not affected up to 4 µl l^{-1}, but decreased at higher levels. Shoot total N content was increased at all levels, mainly due to the increase in free amino acids. Even at atmospheric NH_3 levels, at which the foliarly absorbed NH_3 would cover a limited proportion of N requirement, there was already an enhancement of the nitrogen content of the shoots and roots. Apparently there was no direct regulatory control of and/or interaction between atmospheric and pedospheric nitrogen utilization in *B. oleracea.* It needs to be evaluated to what extent foliarly absorbed NH_3 is used as nitrogen source for growth.

Introduction

Atmospheric N deposition in Europe

NH_3 is a major air pollutant, which accounts for up to 80 % of the total N deposition in central Europe (Fangmeier *et al.*, 1994; Gessler and Rennenberg, 1998; Krupa, 2003). Atmospheric NH_3 pollution is the consequence of intensive farming activities (animal manure and fertilizer use), and to a lesser extent to anthropogenic sources and natural background emissions (Leith *et al.,* 2002; Krupa, 2003; Pitcairn *et al.*, 2003; Erisman and Schaap, 2004). High NH_3 emissions and consequently, excessive N deposition will lead to direct phytotoxic effects, eutrophication and acidification (Stulen *et al.*, 1998; Rennenberg and Gessler, 1999; Krupa, 2003). The toxic effect of NH_3 has often been ascribed to nutrient imbalances due to cation release (Wollenweber and Raven, 1993).

While the impact of atmospheric N deposition on ecosystems such as heathlands (Van der Eerden *et al.*, 1991; Leith *et al.*, 2002; Sheppard and Leith, 2002) and forests (Högberg *et al.*, 1998; Rennenberg and Gessler, 1999; Bassirirad, 2000) has been studied in detail, fewer studies have dealt with its impact on crop plants (Van der Eerden, 1982; Clement *et al.*, 1997). In addition, there is hardly any data available on the contribution of foliar uptake of atmospheric NH_3 to the plant's N requirement for growth (Pérez-Soba and Van der Eerden, 1993; Stulen *et al.*, 1998).

Foliar uptake and metabolism of NH_3

The uptake of NH_3 shows a diurnal variation and is dependent on the water status of the plant, temperature, light intensity, internal CO_2 level and nutrient availability (Hutchinson *et al.*, 1972; Rogers and Aneja, 1980; Van Hove *et al.*, 1987; Husted and Schjoerring, 1996; Schjoerring *et al.*, 1998). The foliar uptake of NH_3 is determined by the stomatal conductance and the internal (mesophyll) resistance to the gas and its uptake via the cuticle surface can be neglected (Krupa, 2003). The internal resistance of the mesophyll cells appears to be the limiting factor for foliar uptake of NH_3 (Hutchinson *et al.*, 1972). The internal resistance to NH_3 is low, since this gas is highly water-soluble and in addition it is rapidly converted into NH_4^+ in the aqueous phase of the mesophyll cells (Fangmeier *et al.*, 1994). NH_3 uptake takes place as long as the atmospheric level exceeds the internal NH_4^+ level (Husted and Schjoerring, 1996).

The NH_4^+ formed in the mesophyll cells may be assimilated by the glutamine synthetase/glutamate synthase cycle (Lea and Mifflin, 1974; Pérez-Soba *et al.*, 1994; Pearson and Soares, 1998). Foliar NH_3 uptake may affect plant metabolism in various ways and result in changes in parameters as metabolic compounds, enzyme activity, root

uptake and plant growth (Pérez-Soba *et al.*, 1994; Gessler and Rennenberg, 1998; Pearson and Soares, 1998). Metabolic changes related to the NH_3 assimilatory capacity of the plant generally lead to an increase in the pool of N-containing metabolites, such as amino acids and total N content (Van Dijk and Roelofs, 1988; Pérez-Soba *et al.*, 1994; Clement *et al.*, 1997; Gessler and Rennenberg, 1998). Visible symptoms, such as black spots and necrosis in the leaves, arise when NH_3 uptake by the shoot exceeds the assimilation capacity of the plant (Van der Eerden 1982; Fangmeier *et al.*, 1994).

Results and Discussion

Impact of NH_3 on growth and N metabolism of Brassica oleracea

The present case study was aimed at investigating the impact of a range of NH_3 levels on growth and N metabolism of *Brassica oleracea* L. Plants were grown on a Hoagland nutrient solution containing 3.75 mM nitrate (for experimental details see Chapter 2). *B. oleracea* was chosen because it is an economically important crop plant with a relatively high RGR, and it is a suitable species because of its preference for nitrate (Pearson and Stewart, 1993) as well as its sensitivity to NH_4^+ (Britto and Kronzucker, 2002). *Brassica* species originate from saline, sulfur-rich environments and are considered to have a high S requirement for growth (Westerman *et al.*, 2000). Therefore, the impact of NH_3 on S compounds was measured as well.

Table 1. Impact of NH_3 on growth of *Brassica oleracea*.

Shoot and root growth (g FW) was calculated by subtracting the final fresh weight from the initial fresh weight. RGR, relative growth rate (g g^{-1} day^{-1}) on a plant basis, determined over a 7 day interval. S/R, shoot to root ratio on a fresh weight basis. DMC, dry matter content (%). Plants (19 days old) were exposed for 7 days. Data represent the mean of 2 experiments, with 3 measurements per experiment with 3 plants in each (±SD). Means followed by different letters are statistically different at $p<0.01$. Statistical analysis was performed by using Student's *t*-test. (For further experimental details see Chapter 2).

[NH_3]	**0 µl l^{-1}**	**2 µl l^{-1}**	**4 µl l^{-1}**	**6 µl l^{-1}**	**8 µl l^{-1}**
Shoot growth	1.90 ± 0.07^{a}	2.04 ± 0.04^{b}	2.45 ± 0.25^{b}	1.78 ± 0.45^{a}	1.69 ± 0.30^{a}
Root growth	0.55 ± 0.20^{b}	0.36 ± 0.06^{b}	0.48 ± 0.08^{b}	0.10 ± 0.07^{a}	0.20 ± 0.11^{a}
RGR	0.20 ± 0.01^{a}	0.20 ± 0.01^{a}	0.20 ± 0.01^{a}	0.16 ± 0.04^{ab}	0.15 ± 0.03^{b}
S/R	3.3 ± 0.6^{a}	4.2 ± 0.3^{a}	4.1 ± 0.4^{a}	5.8 ± 1.1^{b}	5.9 ± 1.5^{b}
Shoot DMC	14.1 ± 1.2^{a}	14.2 ± 1.5^{a}	13.1 ± 1.0^{a}	13.0 ± 1.2^{a}	14.0 ± 0.9^{a}
Root DMC	6.4 ± 1.2^{a}	6.1 ± 0.8^{a}	7.2 ± 0.5^{a}	11 ± 0.4^{c}	9.1 ± 0.9^{b}

Upon NH_3 exposure the shoot biomass production was slightly increased at levels up to 4 µl l^{-1}, whereas it was decreased at levels ≥ 6 µl l^{-1} NH_3. Root biomass production was decreased significantly at 6 and 8 µl l^{-1} NH_3, showing that exposure of the shoot to NH_3 had a negative effect on root growth (in the used experimental conditions, the formation of NH_4^+, by dissolution of atmospheric NH_3 into the nutrient solution, was prevented). Relative growth rate (RGR), calculated on a plant basis was only significantly decreased at 8 µl l^{-1} NH_3. Exposure to 6 and 8 µl l^{-1} NH_3 affected root biomass production relatively more than shoot biomass production, resulting in a higher shoot to root ratio (S/R, Table 1). Shoot dry matter content (DMC) was not affected upon exposure to NH_3, whereas root dry matter content was decreased at 6 and 8 µl l^{-1} NH_3 (Table 1).

Exposure to NH_3 resulted in a substantial increase in shoot total N content at all atmospheric levels (Fig. 1a). This was mainly due to an increase in the soluble N fraction (amino acids, amides and NH_4^+), *viz.* 1.5-fold and 5.6-fold at 4 µl l^{-1} and at 8 µl l^{-1}, respectively, compared to that of the control (0 µl l^{-1} NH_3). Root total N content was only increased at 2 µl l^{-1} NH_3 (Fig. 1a). Shoot nitrate content was increased at all NH_3 levels, but most at 4 µl l^{-1}. Root nitrate content was increased at 2 µl l^{-1}, not affected at 4 µl l^{-1}, and decreased at 8 µl l^{-1} (Fig. 1c). The free amino acid content in the shoot increased with increasing NH_3 levels (8 % and 15 % at 4 µl l^{-1} and 8 µl l^{-1}, respectively), while no effect was observed in the roots (Fig. 1e).

Shoot sulfur content was not affected by exposure to 2 µl l^{-1} NH_3, but decreased at higher levels. Root total sulfur was increased at 2 to 6 µl l^{-1}, and decreased at 8 µl l^{-1} (Fig. 1b). Shoot sulfate content was increased at 2 to 4 µl l^{-1}, and decreased at 6 and 8 µl l^{-1}. Root sulfate content was increased at 2 µl l^{-1}, not changed at 4 µl l^{-1}, and decreased at 6 and 8 µl l^{-1} (Fig. 1d).

The impact of atmospheric NH_3 on total S and sulfate (Fig. 1b, d) can be explained by changes in RGR (Table 1), rather than by a direct effect of NH_3 exposure on S compounds. Noteworthy is the relatively high sulfate content found in this species (Chapter 3).

In the shoot, the organic N/S ratio increased with increasing NH_3 levels, which correlates well with the increase in free amino acid content. Changes in the organic N/S ratio in the root were minor.

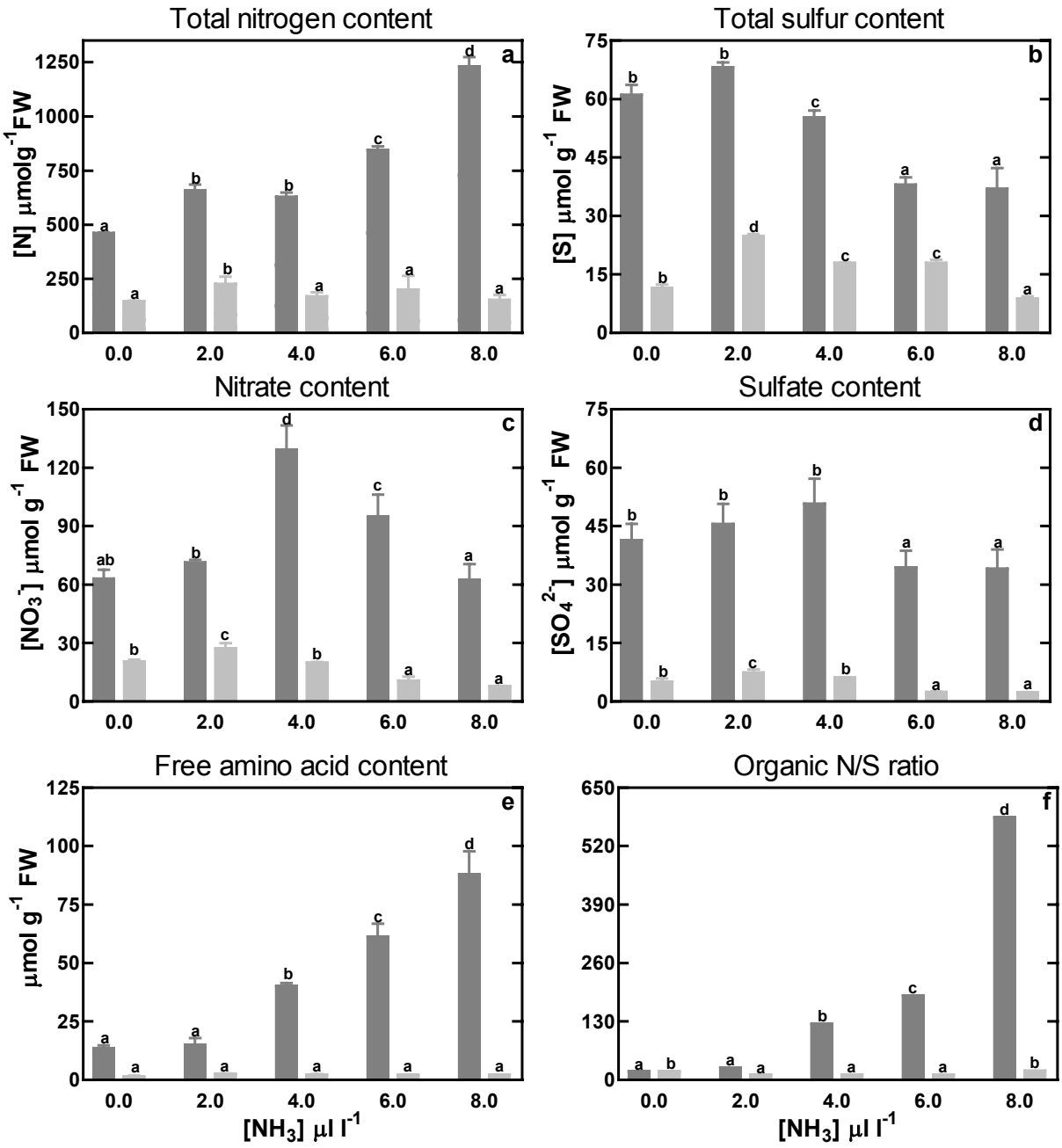

Fig. 1. Impact of NH_3 on N and S compounds in *Brassica oleracea*.

Plants (19 days old) were exposed for 7 days. Shoot data is given in dark-grey bars, root data in light-grey bars. Data on total nitrogen, nitrate and free amino acids contents represent the mean of 2 experiments, with 3 measurements per experiment with 3 plants in each (±SD). Data on total S and sulfate content represent the mean of 3 measurements with 3 plants in each (±SD). The organic N/S ratio was calculated by subtracting the nitrate and sulfate contents from total nitrogen and sulfur contents, respectively. Different letters indicate significant differences at $p<0.01$ (Student's *t*-test). For experimental details see Chapter 2.

Table 2. Net nitrate uptake rate by the root and foliar atmospheric NH_3 uptake by *Brassica oleracea* seedlings. Net nitrate uptake rate (NNUR; μmol g^{-1} FW plant day^{-1}) was measured according to Stuiver *et al.* (1997). NH_3-enhanced N (μmol g^{-1} FW plant day^{-1}) was calculated from the data on RGR and total N presented in Table 1 and Fig. 1, respectively. NH_3 uptake (μmol g^{-1} FW plant day^{-1}) was calculated as the sum of the increase in NH_3-enhanced N and the decrease in NNUR upon exposure to NH_3. Values between brackets represent the atmospheric NH_3 uptake as a percentage of NNUR at 0 μl l^{-1} NH_3. Means followed by different letters are statistically different at $p<0.01$. Statistical analysis was performed by using Student's *t*-test.

[NH_3]	**0 μl l^{-1}**	**2 μl l^{-1}**	**4 μl l^{-1}**	**6 μl l^{-1}**	**8 μl l^{-1}**
NNUR	103 ± 6^{b}	102 ± 8^{b}	74 ± 13^{a}	70 ± 5^{a}	76 ± 7^{a}
NH_3-enhanced N	---	28 ± 4^{a}	38 ± 7^{a}	43 ± 6^{a}	83 ± 7^{b}
NH_3 uptake	---	29(28)	67(65)	76(73)	110(106)

Atmospheric NH_3 – nutrient or toxin?

The requirement for growth of an element can be defined as "the minimum rate of uptake and utilization, which is sufficient to obtain the maximum yield, quality and fitness". Physiologically, the plant's N requirement for growth can be expressed as the rate of N uptake and its assimilation per gram plant biomass produced with time. Similar to observations with S, the N requirement for growth is dependent on the ontogeny and developmental stage of the plant (De Kok *et al.*, 2000, 2002a). In the vegetative stage and at optimal nutrient supply, the plant's N requirement for growth can be estimated from data on relative growth rate (RGR) and plant N content as "$N_{requirement} = N_{content}$ * RGR" (after Williams, 1948). Based on model calculations, Stulen *et al.* (1998) estimated that in theory at an atmospheric level of 2 μl l^{-1} NH_3, the foliar uptake might contribute up to 50 % to the N requirement of plants growing at a RGR of 0.2 g g^{-1} day^{-1} (may vary between species differing in N requirement). From the current experimental data the foliar uptake of NH_3 at the various atmospheric levels and its possible contribution to the plant's N requirement could be estimated. NH_3 exposure resulted in enrichment of the total N content of the plant (Fig. 1), which could analogue to the formula of William (1948) be defined as an atmospheric NH_3-enhanced N and calculated as follows:

NH_3-enhanced N at $[NH_3]$ =

$$\{((RGR * N_{content}) \text{ at } [NH_3] - ((RGR * N_{content}) \text{ at } 0 \text{ μl } l^{-1} NH_3)\}$$

where $[NH_3]$ represents the NH_3 level plants have been exposed to. The NH_3-enhanced N is expressed as μmol N g^{-1} FW plant day^{-1}.

The net nitrate uptake rate (NNUR) was decreased upon exposure to 4 to 8 µl l^{-1} NH_3 (Table 2). If one would assume that the observed decrease in NNUR would be due to a partial transfer of the plant from pedospheric nitrate to foliarly NH_3 taken up as nitrogen source for growth, then the sum of the NH_3-enhanced N and the decrease in NNUR (µmol N g^{-1} FW plant day^{-1}) would represent the total atmospheric NH_3 uptake by the plant. On basis of these estimations the possible contribution of the atmospheric NH_3 uptake by the foliage at 2, 4, 6 and 8 µl l^{-1} NH_3 could have accounted for 28, 65, 73 and 106 % of the plant's N requirement, respectively. However, from the observations that there was already an enrichment in the total N content upon exposure to a level as low as 2 µl l^{-1} NH_3 one may conclude that at a sufficient nitrate supply to the roots either the foliarly absorbed NH_3 was hardly utilized for structural growth or there was a poor shoot to root signaling in tuning the rate of metabolism of the absorbed reduced nitrogen in the shoot to the nitrate uptake by the root. This is in contrast with observations upon exposure of *B. oleracea* for instance to the sulfurous air pollutant H_2S, where there was a strong interaction between the rate of uptake and metabolism of H_2S in the shoot and the uptake of sulfate by the roots (Westerman *et al.*, 2000). This might be explained by differences in the factors determining the internal resistance to NH_3 and H_2S. In contrast to NH_3, where the internal resistance is determined by its high solubility and dissociation in the aqueous phase of the mesophyll cells, that of H_2S is largely determined by its rate of metabolism (De Kok *et al.*, 2002b).

Conclusions

The present results demonstrated that at atmospheric levels up to 4 µl l^{-1}, NH_3 appeared not to be toxic to *B. oleracea*. Higher NH_3 levels had a negative effect on growth, likely due the negative effect of high intracellular NH_4^+ levels, or possible formed metabolites during cellular metabolism. The current NH_3 levels in field conditions range from 0.03 µl l^{-1} to 1.2 µl l^{-1}, the latter as a peak level (Stulen *et al.*, 1998). It is unlikely that these levels would negatively affect growth of *B. oleracea* under field conditions. This is in agreement with the observations of Van der Eerden (1982), who exposed various plant species, including *B. oleracea,* to 0.8-1.4 µl l^{-1} NH_3 and found no differences in growth. Apparently *B. oleracea* is able to cope with even higher levels of NH_3, most likely because of its higher nitrogen requirement and high RGR.

It remains to be questioned to what extent foliarly absorbed NH_3 is used as nitrogen source for growth, if the nitrogen supply to the roots is sufficientor insufficient. Even at atmospheric NH_3 levels (*e.g.* 2 µl l^{-1}), which only would cover a limited proportion of the

N requirement if used as nitrogen source, there was an enhancement of the nitrogen content of the shoots and roots. Apparently there was no direct regulatory control of and/or interaction between atmospheric and pedospheric nitrogen utilization in *B. oleracea.*